UNIVERSITI PUTRA MALAYSIA

TOXICITY AND IMMUNOSUPPRESSIVE EFFECTS OF DIAZINON IN GRASS CARP, CTENOPHARYNGODON IDELLA (CUVIER AND VALENCIENNES)

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By

REZA POORGHOLAM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of Requirements for the Degree of Doctor of Philosophy

May 2005

DEDICATION

WITH LOVE AND APPRECIATION TO:

My parent: Rajabali Pourgholam and Sedigheh Sajoodi

My wife: Sekineh Dashti

My sons: Hamzeh, Mohebali and Mohammadali

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

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Chairman: Associate Professor Hassan Hj. Mohd Daud, Ph D

Faculty : Veterinary Medicine

Grass carp is one of the valuable warm water fish species that is currently being cultured in polyculture system in Iran. Despite of large scale grass carp farming in the East Asian countries, only minimum data is available concerning the fish immune system and the effect of organophosphate chemicals on the fish immune response. Diazinon is one of the major organophosphate pesticides currently used in Northern and Southern part of Iran. Unfortunately, these areas are also the main regions for grass carp culture and there are regular reports of the disease outbreaks particularly in the provinces of Gilan and Khozestan. In addition, previous studies has indicated that Aeromonad septicemias was one of main factor in the high mortality occurrences in the grass carp, in particular whenever the fish immune system seems to be suppressed by some toxicants.

The specific objectives of this study were: (i) determination of 96-h LC_{50} diazinon in grass carp; (ii) purification and partial characterization of grass

carp IgM; (iii) assessment of some humoral and cellular immunoresponses of non- immunized and immunized grass carp, following exposure to diazinon.

In this study, LC_{50} of diazinon at 96 hour in grass carp was determined to be 15.13 mg/L. The examination of hematological and tissue enzyme parameters indicated that diazinon at sublethal levels had caused an effect similar to anemia. In addition, a significant decrease of lymphocytes values and significant increase of PMNs values were observed. There were also significant and insignificant changes in some blood parameters such as monocytes and myelocytes counts, MCH, MCHC, AST, ALP, ALT and LDH values at different days of post exposure of diazinon. Such fluctuations indicated that fish hematopoietic tissues were in stress and were in constant struggles to maintain normal condition.

The pathological effects of diazinon on the liver, kidney, spleen, gills, and nostrils of grass carp examined under light and electron microscope, showed that diazinon caused severe damage to the cell structure such as congestion of blood vessels, haemorrhage, cellular infiltration, pyknosis of cells nuclei, vacuolar degeneration and general necrosis in the tissues of kidney, spleen and liver. There were also degenerative changes of interstitial tissue, detachment of tubular basement membrane in kidney. In the gills, hyperplasia and fusion of secondary lamellae, separation and sloughing of epithelium from the underlying basement membrane were also observed.

In the lysozyme study it was indicated that grass carp reacted to diazinon by raising the level of lysozyme in tissues of spleen and kidney and also in

serum of Aeromonas-immunized fish exposed to diazinon and control positive (immunized only), as compared to control negative (non-immunized and not exposed to diazinon). However, the level of lysozyme in immunized fish exposed to diazinon is lower than control positive that indicated the depressive effect of toxicant on fish immune system. Analysis on data of leucocytes chemiluminescent response indicated that cellular factors of fish immunity following immunization with *A. hydrophila* and also under influence of diazinon, responded by changing its functional activities, as evidenced by a high chemiluminescent response in both of immunized fish exposed to diazinon and control positive groups as compared to the control negative group. However, the level of chemiluminescent response in immunized and exposed group was insignificantly lower than control positive group that indicated the negative effect of diazinon on fish immune system. In summary, all of the above findings proved the immunosuppressive effect of diazinon on nonspecific immune system of grass carp.

The level of IgM in serum of normal grass carp was found to range from 3 to 4 mg/ml. Estimation of molecular weight of grass carp IgM was performed using three different methods. Affinity chromatography method gave the approximate values of about 480 and 640 KDa in SDS-PAGE, while gel chromatography and ion-exchange chromatography methods showed an identical molecular weight with an approximate value of 490 KDa.

In antibody study, the titers of immunized fish were significantly higher than immunized fish exposed to diazinon. In addition, a strong positive correlation

was also demonstrated between the results of ELISA and agglutination titers. These observed results confirmed the immunosuppressive effect of diazinon on specific immune system of grass carp.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

Ketoksidan dan Kesan Imunotindas Diazinon pada Ikan Kap Rumput, *Ctenopharyngodon idella* (Cuvier and Valenciennes)

Oleh

REZA POORGHOLAM

Mei 2005

Pengerusi: Profesor Madya Hassan Hj. Mohd Daud, Ph D

Fakulti : Perubatan Veterinar

Kap rumput adalah salahsatu spesis ikan air suam yang bernilai tinggi buat masa ini di kultur dalam sistem polikultur di Iran. Meskipun penternakan ikan kap rumput dijalankan secara besar-besaran di negara Asia Timur, tetapi cuma terdapat data minima mengenai sistem keimunan ikan dan kesan bahan kimia organofosfat ke atas ransangan imun. Diazinon adalah racun serangga utama pada ini digunakan di Utara dan Selatan Iran. Walau bagaimanapun, kawasan ini adalah juga kawasan utama untuk ternakan ikan kap rumput dan terdapat laporan pencetusan penyakit ikan di daerah Gilan dan Khozestan. Tambahan lagi, kajian terdahulu telah menunjukkan bahawa septisemia Aeromonads adalah Salah satu faktor utama yang menyebabkan kejadian kematian yang tinggi pada ikan kap, terutamanya apabila sistem keimunan ikan tertindas oleh bahan-bahan toksid.

Objektif spesifik kajian ini adalah : (i) penentuan LC_{50} diazinon pada 96-jam pada ikan kap rumput ; (ii) permurnian dan pencirian separa IgM kap rumput;

(iii) penilaian beberapa ciri ransangan imun humoral dan selular ikan kap yang diimun dan tak diimun, selepas didedahkan kepada diazinon.

Di dalam kajian LC_{50} diazinon pada 96-jam pada ikan kap rumput, nilainya ditentukan pada 15.13 mg/L. Pemeriksaan parameter hematologikal dan tisu enzim menunjukkan diazinon pada aras subletal telah menyebabkan kesan sama seperti anemia. Di samping itu terdapat penurunan bererti dalam nilai limfosit dan peningkatan bererti dalam nilai PMN. Disaksikan juga perubahan yang bererti dan tak bererti dalam nilai-nilai parameter darah yang lain seperti bilangan monosit dan mielosit dan nilai-nilai MCH, MCHC, ALP, ALT dan LDH. Perubahan-perubahan tersebut menunjukkan tisu hematopoietik adalah dalam situasi tindasan dan sentiasa berjuang untuk berada dalam keadaan normal.

Kesan patologikal diazinon pada hepar, ginjal, limfa, insang dan rongga nasal yang dilihat dibawah mikroskop cahaya dan elektron menunjukkan bahawa diazinon telah menyebabkan kecederaan teruk pada struktur tisu seperti kongesi saluran darah, hemoraj, penyusupan sel, piknosis nukleus sel, degenerasi perlompangan dan nekrosis am di dalam tisu ginjal, limfa dan hepar. Terdapat juga perubahan degeneratif pada tisu perantaraan dan perlucutan tapak membran sel tubular ginjal. Pada insang, hiperplasia dan percantuman lamela skunder, perpisahan dan penghakisan epitelium daripada tapak membran juga dapat dilihat.

Di dalam kajian lisozim, ia menunjukkan bahawa kap rumput bertindakbalas terhadap diazinon dengan meningkatkan aras dalam tisu limfa dan ginjal dan juga serum ikan yang diimunkan selepas pendedahan kepada diazinon dan dalam kumpulan kawalan positif (diimunkan), jika dibandingkan dengan kumpulan kawalan negatif (tidak diimunkan dan tidak didedahkan kepada diazinon). Walau bagaimanapun, aras lisozim dalam ikan terimun terdedah pada diazinon adalah lebih rendah dari kumpulan kawalan positif yang mana membuktikan bahawa kesan tindas toksikan terhadap sistem imun ikan. Analisis terhadap data ransangan pendaflorkimia leukosit, menunjukkan bahawa faktor selular imuniti ikan selepas imunisasi dengan *A. hydrophila* dan di bawah pengaruh diazinon, bertindak dengan menukar fungsi aktiviti yangmana dapat dilihat pada gerakbalas tinggi dalam pendarfluorkimia dalam ikan yang diimun dan terdedah kepada diazinon dan kumpulan kawalan positif dibanding dengan kumpulan kawalan negatif. Walau bagaimanapun, aras gerakbalas pendarfluorkimia di dalam kumpulan ujian adalah lebih rendah, walaupun tidak bererti, menunjukkan kesan negatif diazinon terhadap sistem imun ikan. Kesimpulannya, penemuan membuktikan kesan imunotindas diazinon terhadap sistem imun tak spesifik ikan kap rumput.

Manakala aras IgM dalam serum ikan kap rumput normal berada dalam renj 3-4 mg/L. Anggaran berat molekul IgM kap rumput telah dibuat menggunakan tiga metod yang berbeza. Metod kromatografi affiniti memberi nilai anggaran 480 dan 640 KDa dalam SDS-PAGE, sementara kromatografi

gel dan kromatografi tukaran-ion menunjukkan berat molekul yang identikal iaitu bernilai anggaran 490 KDa.

Dalam kajian antibodi, titer dalam ikan yang diimun adalah lebih tinggi dan bererti dari ikan yang diimun dan didedahkan kepada diazinon. Tambahan lagi, korelasi positif yang kuat telah ditunjukkan di antara keputusan ELISA dan titer agglutinasi. Penemuan membuktikan kesan imunosupresif diazinon terhadap sistem imun spesifik ikan kap rumput.

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I certify that an Examination Committee met on 12th May 2005 to conduct the final examination of Reza Poorgholam on his Doctor of Philosophy thesis entitled "Toxicity and Immunosuppressive Effects of Diazinon in Grass Carp, *Ctenopharyngodon idella* (Cuvier and Valenciennes)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

REZA POORGHOLAM

Date : 20 Jul 2015

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